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(71) Applicants

Patrick Douglas Shaw

Stewart,

Linplum House,

Haddington,

East Lothian,

Scotland.

(72) Inventors

Patrick Douglas Shaw

Stewart

(54) Combining chemical reagents

(57) The invention is a method of combining chemical reagents and comprises the steps of:

1) Dispensing the reagents into a conduit system in the form of discrete volumes or droplets separated from each other by an inert immiscible liquid, and

2) Moving the inert liquid and discrete droplets in such a manner that predetermined discrete volumes

coalesce with each other, thereby combining the reagents. The preferred apparatus for carrying out the method comprises a plastic block (2) with indentations (3) which is clamped against a glass plate (4) forming closed conduits which are connected to reservoirs of light mineral oil, or silicon oil, by thin tubing (9) and valves (10). All the conduits are initially filled with the oil, then the second and fourth tubes are used to

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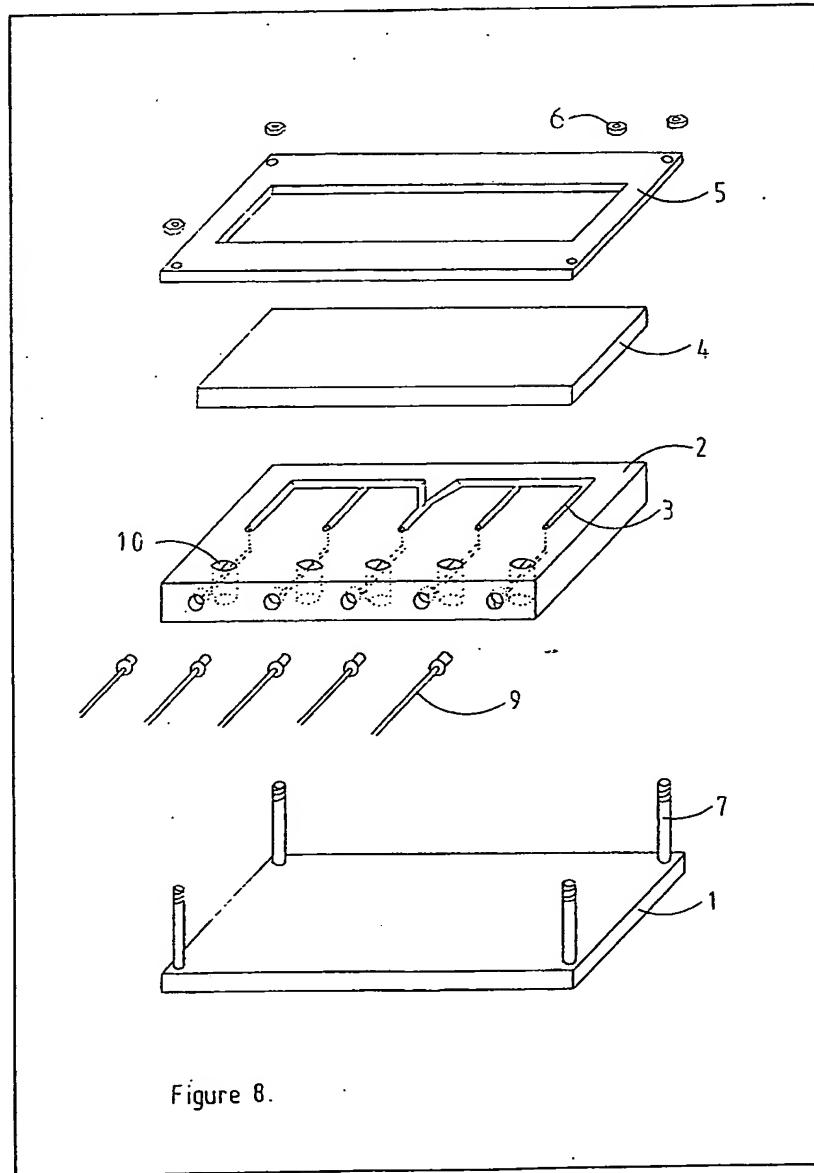


Figure 8.

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introduce two reagents, which are separated into two smaller volumes at the 'T' junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit.

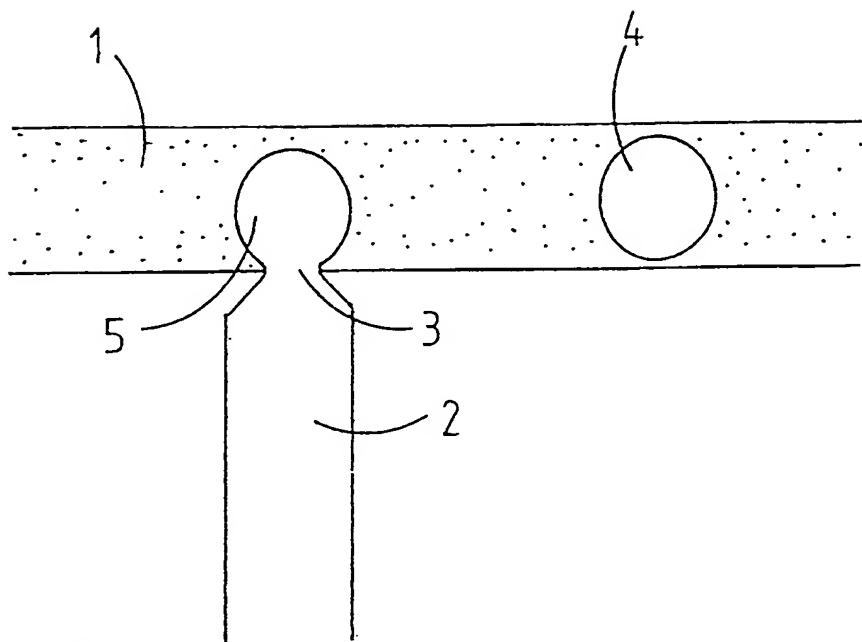


Figure 1.

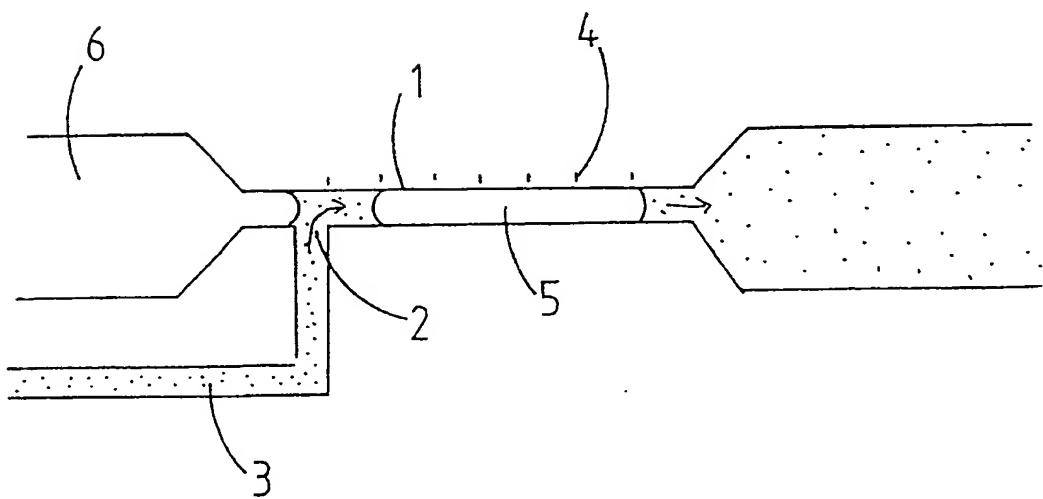


Figure 2.

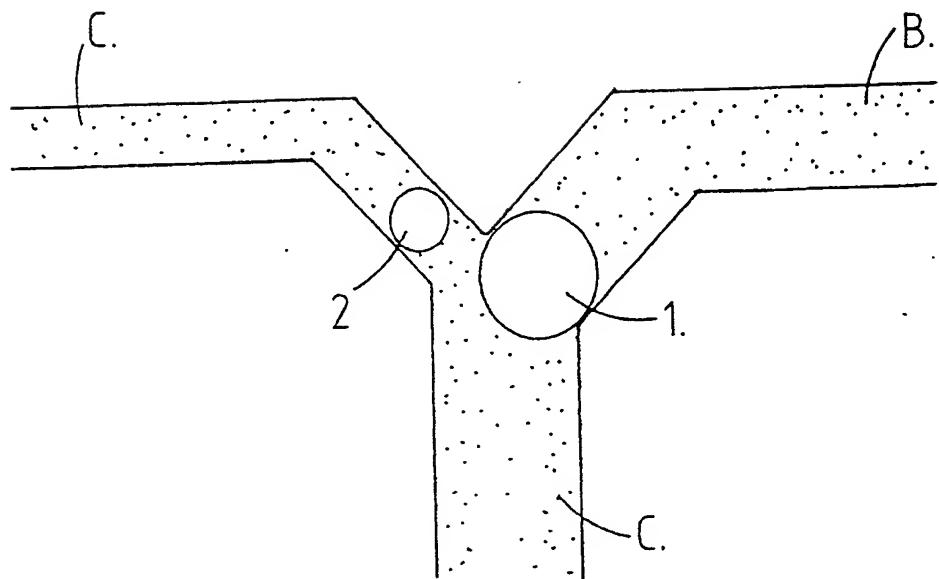


Figure 3.

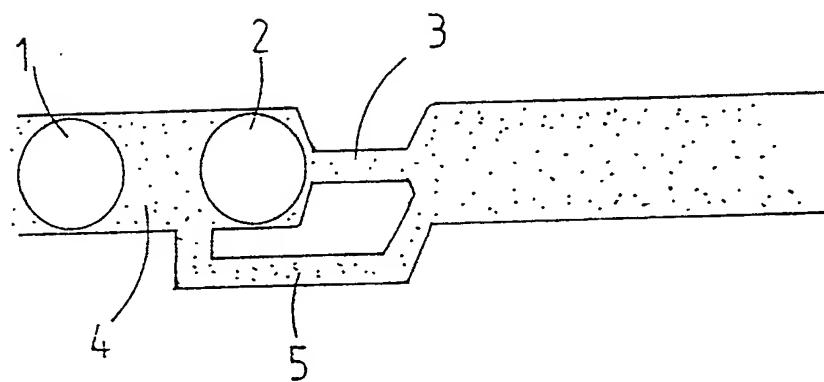


Figure 4.

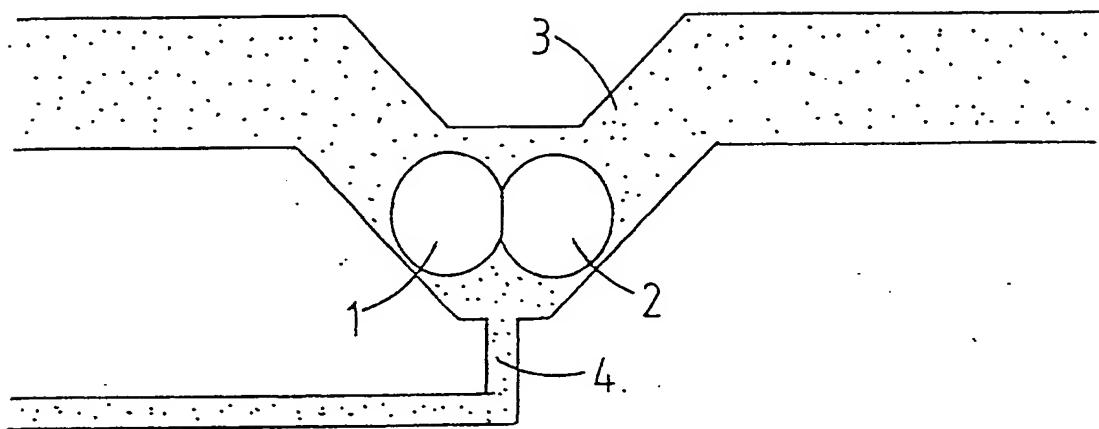


Figure 5.

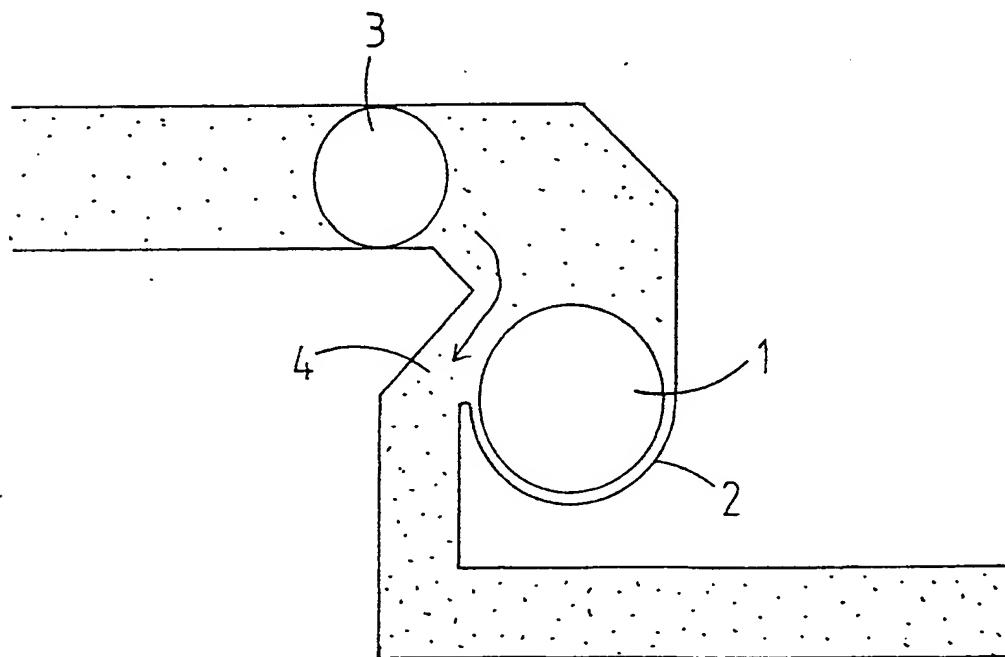
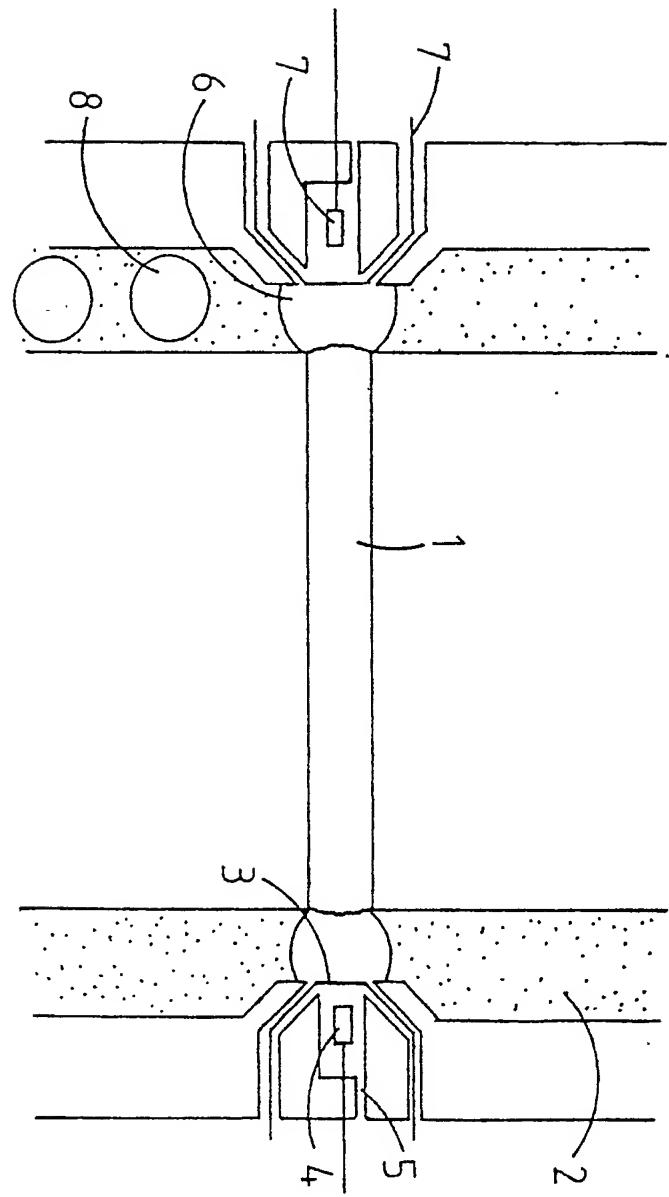


Figure 6.

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Figure 7



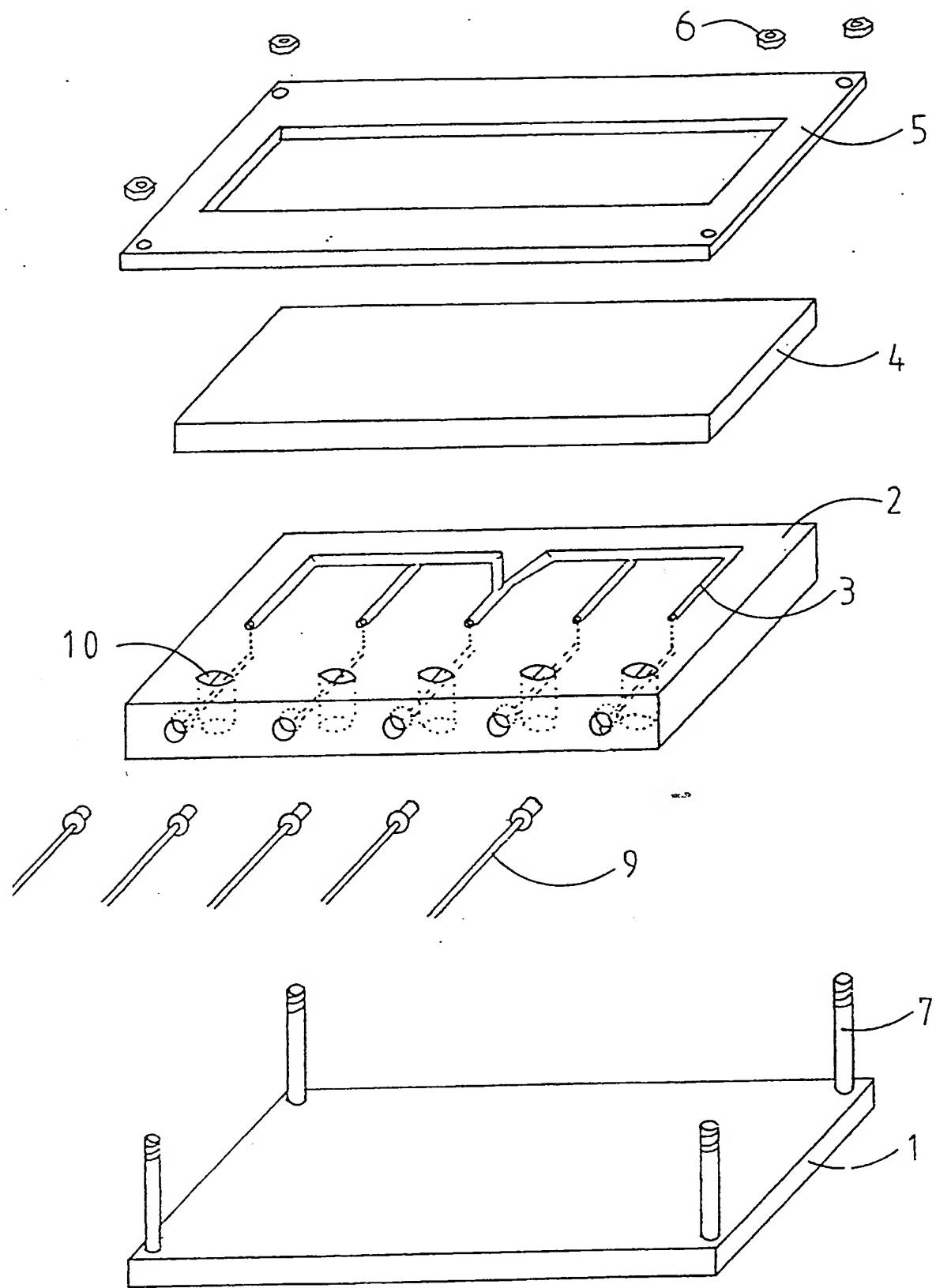


Figure 8.

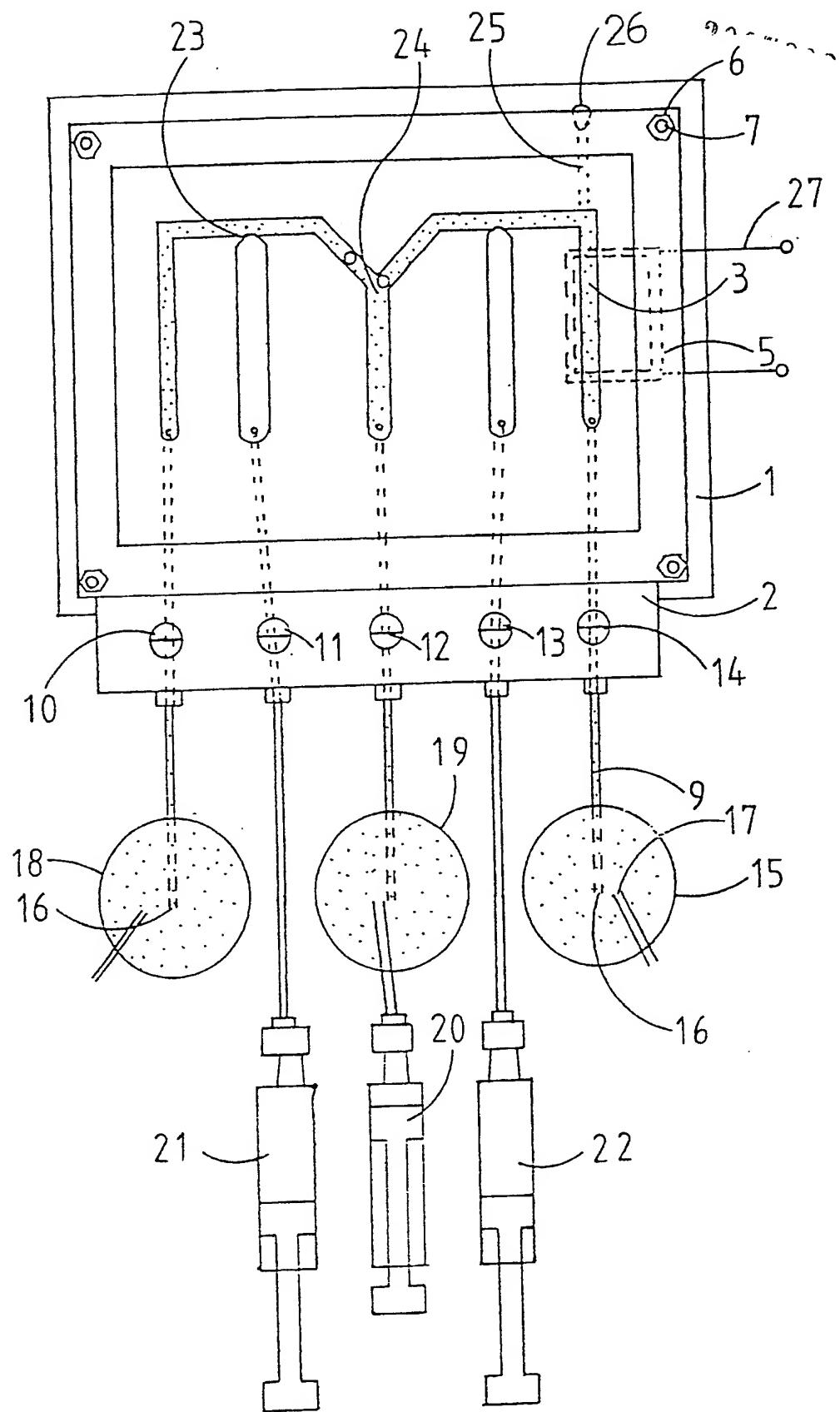


Figure 9.

SPECIFICATION

Droplet Reactor

5 This invention relates to a method for combining chemical reagents and to apparatus for use therein.

The method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents and comprises the

10 steps of:

1) dispensing two or more reagents, optionally in a solvent phase, into a conduit system in the form of discrete volume being separated from each other by an inert immiscible liquid, and

15 2) moving the inert immiscible liquid and discrete volumes through the conduit system in such a manner that that predetermined discrete volumes coalesce with each other thereby combining the reagents.

20 The system is particularly suited to the manipulation of microscopic quantities of reagents, with volumes of less than one microlitre, but larger quantities of up to several litres could also be used. In any application, the particular advantages of the

25 system will be:

1) the reactants may be manipulated quickly and efficiently, and

2) losses of material by spillage or adhesion to glassware, and contamination are substantially eliminated and

3) automation of the process is simple and less complex than in conventional methods.

The reagents should be either liquids, or gases which can be condensed at the operating temperature and pressure of the apparatus, or solids or gases dissolved in a suitable solvent. This reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase. Suitable

40 carrier phases include mineral oils, water, light silicones, or Freons. The carrier phase may also be a mixture of several substances, and preferably has approximately the same density as the reagents.

45 Surface acting agents may also be included in the carrier and/or reagent phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioxy, succinate Teepol, and Triton-X-100.

The liquid components will be contained in a

50 system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents, and to be divided into small droplets. The cross-section of the tubes is generally of the same order of magnitude as the cross-section of the droplets which are to be used in them. The carrier phase may be moved by any suitable means e.g. pumps and pistons. The pistons can conveniently be moved by screw threads operated by electric

60 motors, optionally controlled electronically by microprocessor technology.

For aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon

interface.

Figure 1 of the accompanying drawing illustrates one method of breaking of droplets of a predetermined volume from a larger reservoir of reagent.

70 The reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier

75 phase, down the tube.

The volume of the droplet (4) produced, can be determined by three methods:

(a) The piston responsible for pushing the reagent through the opening is moved a measured distance. When this movement is complete, the droplet is broken off by a relatively fast but short-lived current in the tube (1).

80 (b) Alternatively, if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of

90 the two currents.

(c) The volume of the droplet before separation is estimated visually by a microscope or telescope with a graticule eye-piece. When the correct volume is achieved it is broken off by a current of carrier

95 phase.

Figure 2 illustrates another method of forming droplets. In this case the reagent (6) is passed into a tube of considerably narrower bore (1) than the cross-section of the droplets to be produced. The

100 tube is graduated relative to the opening (2) of a side-arm (3). The reagent is passed into the tube to a certain graduation (4), whereupon carrier phase is introduced from the side-arm, thus breaking off a droplet of the required size (5).

105 The volume of a droplet may be measured both before and after it is separate by inspecting the droplet with a microscope with a graduated eye-piece.

Figure 3 illustrates a method of coalescing droplets, which may be of different sizes. The coalescence is effected at a site where two ducts, A and B in Figure 3, merge to form a single duct C, preferably in a Y-configuration as shown.

A current from duct B to duct C is produced which

115 carries a droplet (1) to the site for coalescence. When

the surface of this droplet protrudes into the space where ducts A and B merge, the valves feeding duct B are closed. A current from duct A to duct C now carries a second droplet (2) past the first, and in

120 doing so the two droplets are pressed together, and coalesce to form a single droplet.

If either droplet is smaller than the other, then the duct through which it emerges should be correspondingly narrower. All ducts preferably have

125 approximately the same width as the droplets to be used in them.

Figure 4 illustrates another method of coalescing droplets. The two droplets (1) and (2) are passed down a tube until the first reaches, and is restrained

130 by a constriction (3). The carrier fluid between the

droplets (4) is diverted by a by-pass (5) opening into the region between the droplets, and the droplets are pressed together and coalesce. In some circumstances it is possible to achieve this without a by-pass 5 or side-arm by having a constriction with an irregular or flattened cross-section. This prevents the first droplet from completely blocking the constriction, and allow the carrier fluid between the droplets to pass. If a by-pass is used, it should be sufficiently 10 long and/or narrow to offer a greater resistance to the carrier fluid than the constriction itself. Alternatively the fluid could be sucked from between the droplets by means of a side-arm leading to a separate piston, valve or suction generating component. 15

Figure 5 illustrates a method of coalescing droplets under gravity. Two or more droplets (1) and (2) are passed into a U-shaped tube (3), where they are maintained until they coalesce. It is convenient to 20 use a carrier phase for carrying the droplets to the U-tube which contains a surfactant agent which prevents merging, and to introduce a small quantity of a immiscible carrier phase containing a surfactant agent which encourages merging by means of a 25 side-arm (4), when the droplets are in position in the U-tube.

Figure 6 illustrates a method which relies on pressure and gravity. The first droplet (1) falls into a cavity (2) where it is constrained by gravity while the 30 fluid between the droplets is removed by passing through a nearby constriction (3). The second droplet cannot also fit into the cavity, and is sucked towards the constriction, and is thus pressed against the first droplet with which it coalesces. 35 Figures 5 and 6 illustrates the situation where the droplets are denser than the carrier phase. Inverted versions may be used if the droplets are less dense than the carrier phase.

The various movements of the fluids are produced 40 by connecting the components shown to pistons, pumps or to reservoirs containing either compressed gas or partial vacuums. Control is achieved by means of conventional valves.

Regions of the device can be heated by electric 45 circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature. Solid state heat pumps can be used to cool other regions both for incubation at low temperatures and to allow the storage in reservoirs 50 of unstable reagents or samples.

Heating and cooling can also be accomplished by a circulated fluid.

After coalescence the constituents of two droplets can be mixed by moving the resultant droplet up and 55 down a duct, or if necessary by passing it through a constriction once or several times.

Ports can be provided to allow droplets to be removed by sucking them into a syringe needle.

The droplets can also be sucked into a tube and 60 transferred to any standard instrument or chemical or biochemical analysis. Alternatively parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis. For example ducts can be 65 formed with two plain transparent walls to form the

sample chambers of spectrophotometers. One plain transparent wall opposite a reflecting surface could also be used.

Ducts can also be packed with the materials used 70 for electrophoresis and chromatography.

Figure 7 shows a duct which is adapted for electrophoresis. The electrophoretic duct (1) is packed with the electrophoretic material. the sample to be separated by electrophoresis is introduced by a duct 75 (2) and merges on one side with the surface of the electrophoretic material (e.g. agarose, polyacrylamide or cellulose), and with a semipermeable membrane (3) on the other. This semipermeable membrane encloses a chamber filled with buffer and 80 containing an electrode (4) and provided with a gas vent (5) if required. Another droplet (6) is merged to the other end of the electrophoretic material and to another semipermeable membrane and electrode assembly (7). A current is passed between the 85 electrodes which causes the first fragments to migrate across to the receiving droplet (6). This droplet is exchanged for another (8) after a period, allowing the collection of a series of fractions in droplets.

90 The ability to handle small samples also makes the system suited for use with high pressure liquid chromatography. The sample can be connected to a conventional H.P.L.C. system by fine bore tubing.

The progress of the droplets can be automatically 95 monitored by detecting fluctuations in the light propagated through the tube as the droplets pass. When two droplets merge the light scattered from a beam passing through both droplets will suddenly decrease. Windows in an opaque coating of the tube 100 can be used, or in cases where information is required from any sites, optic fibres could carry information in the form of light to a central detector. In such an automatic system the valves and pistons used would also be operated electrically, under 105 microprocessor control.

The preferred method of construction of the apparatus is to produce indentations of the appropriate configuration in the surface of a plate and to clamp the plate against another plate, producing 110 closed ducts.

The second plate may have a planar surface or may have indentations, either in the mirror image of the first plate's or in a different configuration. Also it is possible to produce three dimensional configurations, where ducts cross over one another, by using 115 three or more plates.

Preferably at least one of the plates should be transparent, e.g. glass or perspex, for the operator to visually inspect the procedures. The other plates 120 may be Kel-F or Teflon (materials suitable for use with aqueous droplets) or metal, glass, polyvinylchloride, polypropylene etc.

Indentations in a sheet of glass can be produced by masking all areas of the surface with wax and 125 etching with hydrofluoric acid. Plastics and metals can be either etched, moulded or machined.

Electrical contacts can be produced by depositing a metal coating in the desired configuration on either of the blocks, either by selectively etching a coating 130 or by masking regions of the block during the

deposition process.

Figure 8 represents an exploded view of one embodiment of an apparatus in accordance with the invention. This embodiment is capable of mixing 5 predetermined volumes of two aqueous reagents under sterile conditions.

The device comprises a steel base (1) supporting a Teflon block (2) in which U-shaped indentations (3) of the configuration shown have been machined. A 10 sheet of siliconized glass (4) is pressed against this block by a clamp (5) which is tightened by nuts (6) and bolts (7). The five branches of the U-shaped ducts lead to five stop-cock valves (10). The middle and outer pair of these ducts are connected by teflon 15 tubing (9) to reservoirs of the carrier phase, which in this case is white spirit. The remaining two branches are connected to glass syringes, each containing one of the two reagents to be mixed.

Figure 9 represents a plan view of the device of 20 Figure 8 and uses the same reference numerals. Reservoirs (15) and (18) are half-filled with white spirit with the end of the tubing (16) opening under the surface of the white spirit, while the end of an air vent (17) opens above the surface. A syringe containing 25 air (20) is connected to reservoir (19) above the surface of the white spirit. The ports serving the valves (11) and (13) lead to two syringes (21) and (22) containing the two reagents. The ducts are initially filled with white spirit.

30 The operating sequence for one cycle is as follows:

1. Valves (10), (11) and (12) are opened. Valves (13) and (14) are closed.
2. Syringe (21) is moved in until reagent reaches 35 constriction (23).
3. Syringe (21) is moved further by the correct amount, pushing a known volume of reagent through the constriction (23).
4. Valve (11) is closed.

40 5. Syringe (20) is moved out until the volume protruding through the constriction is broken off and moves as a droplet towards the Y-Junction (24). Valve (10) is closed before the droplet reaches this junction.

45 6. Valves (13) and (14) are opened and a similar sequence of events produces a droplet of the reagent from syringe (22).

50 7. Syringe (20) moves out until the droplet originating from syringe (22) protrudes into the Y-Junction. Valve (14) is closed. The positions of the two droplets at this stage are illustrated in Figure (9). 8. Valve (10) is opened and syringe (20) moves out until the droplet of the reactant from syringe (21) moves into the Y-Junction (24), where it is pressed 55 against the other droplet causing the two droplets to coalesce.

9. If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using a thermostatically controlled heating coil (27). The droplet can be discarded by passing 60 it into a reservoir (19) of white spirit.

If the droplet is to be analysed using external instrumentation, it can be moved to a site opposite port (25), all valves closed, plug (26) removed and 65 the droplet impaled and removed by a needle and

syringe.

10. Syringe (20) is disconnected, emptied of air, and reconnected, leaving the device primed for another cycle of operation.

70 More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible. It is possible to grow microbes within the droplets, without risk of contamination.

75 In place of droplets larger segments occupying some considerable length of duct can be used. Contamination can be further reduced by passing droplets or larger segments of a cleaning liquid which is miscible with the reactant droplets through 80 the system prior to the passage of the droplets or larger segments of reactant.

This invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize 85 very small quantities, such as forensic and recombinant DNA work.

CLAIMS

90 1. A method of combining chemical reagents by:
 1. dispensing two or more reagents, optionally in a solvent phase into a conduit system in the form of discrete volumes of liquid, each discrete volume being separated from each other by an inert immiscible liquid and,
 2. moving the immiscible liquid and discrete volumes through the conduit system in such a manner that predetermined discrete volumes coalesce with each other thereby combining the 95 reagents.

100 2. A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.

105 3. A method as claimed in claim (1) in which the discrete volumes of chemical reagents are large enough to be elongated by the walls of the conduits.

110 4. A method as claimed in claims (1), (2) and (3) in which each of the discrete volumes of chemical reagents is formed by the passing of the chemical reagent from a conduit into one or both of two conduits which have previously been filled with the inert immiscible liquid and which converge with the conduit containing the chemical reagent, followed 115 by the moving of the inert immiscible liquid causing a segment of the chemical reagent to become separated from the chemical reagent which remains in the duct from which the chemical reagent was introduced.

120 5. A method as claimed in claim (4) in which the conduit from which the chemical reagent is introduced has a constriction at the point where it meets the two conduits which were previously filled with inert immiscible liquid, which allows the segment of 125 the liquid to be mixed to be separated by a less powerful movement of the inert immiscible liquid, and with a more predictable volume.

130 6. A method as claimed in claim (4) in which the volume of the chemical reagent is estimated before separation by assuming said volume is spherical and

measuring the diameter of such a sphere with a microscope or telescope with a graduated eyepiece, after which an adjustment of said volume may be made and said volume separated.

5 7. A method as claimed in claim (4) in which the volume of the chemical reagent that is passed into the conduits which were previously filled with inert immiscible liquid is sufficiently large that said volume occupies a length of said conduits, which 10 length may be used as a measure of said volume prior to the adjustment and separation of said volume.

8. A method as claimed in claim (4) in which the volume of the chemical reagent that is passed into 15 the conduits that were previously filled with inert immiscible liquid is determined by displacing the required volume out of the conduit containing the chemical reagent by moving a syringe or piston a known distance, or by means of a well calibrated 20 pump.

9. A method as claimed in any preceding claim in which the passage of a segment of chemical reagent is preceded by the passage of a segment of cleansing liquid which is miscible with the reagents, but 25 immiscible with the inert immiscible liquid.

10. A method as claimed in any previous claim in which surface acting chemical agents are dissolved in the chemical reagents or in the immiscible liquid or in both.

30 11. A method as claimed in claim (1) in which the discrete volumes of the chemical reagents are replaced by discrete volumes of liquids containing suspended biological micro-organisms.

12. A method of combining chemical reagents as 35 claimed in claim (1) substantially as herein described with reference to Figures 1, 2, 4, 5 and 6 of the accompanying drawings.

13. A method of combining chemical reagents as claimed in claim (1) substantially as herein described 40 with reference to Figures 3, 7, 8 and 9 of the accompanying drawings.

14. An apparatus for carrying out any of the methods carried in previous claims comprising two plates held in face to face contact, in which one or 45 45 both plates possess a set of indentations on the surface to be mated such that closed conduits are formed, said conduits being in communication with reservoirs for at least 2 reagents and an immiscible liquid.

50 15. An apparatus as claimed in claim (14) in which valves, pumps, syringes, pistons or flexible sheets or tubes are used to produce and control the movements of the liquid components of the system.

16. An apparatus as claimed in claim (14) in 55 which at least one of the plates is transparent.

17. An apparatus as claimed in claims (14), (15), or (16) having three or more plates which allows the construction of overlapping and crossing over conduits.

60 18. An apparatus for carrying out the method claimed in claim (1) in which tubular elements are joined so as to form the conduits for the introduction of discrete volumes of at least two chemical reactants, and zones where at least three conduits meet, 65 which zones allow the coalescence of said discrete volumes of chemical reagents.

19. An apparatus as claimed in claims (14) or (18) which possesses one or more zones for the coalescence of discrete volumes of chemical reagents, each 70 zone being characterized by the meeting of at least three conduits, preferably in a 'Y' or 'T' configuration in the case of three conduits and in which each conduit may be of a different width.

20. An apparatus as claimed in claims (14) or (18) 75 which possesses one or more zones for the coalescence of discrete volumes of chemical reagents, each zone being characterized by a constriction in a conduit, which constriction is bypassed by a conduit which has a diameter comparable to that of the 80 constriction and which runs from shortly before the constriction to shortly after it.

21. An apparatus as claimed in claims (14) or (18) which possesses one or more zones for the coalescence of discrete volumes of chemical reagents, each 85 zone being characterized by a depression in which a discrete volume of chemical reagent is held by gravity while another discrete volume is moved by the inert immiscible liquid towards a nearby opening, which movement causes the discrete volumes 90 to become pressed against each other allowing coalescence.

22. An apparatus as claimed in (14) or (18) possessing conduits with hydrophobic walls for use with aqueous or polar chemical reagents.

95 23. An apparatus as claimed in (14) or (18) possessing conduits with hydrophilic walls for use with hydrophilic or non-polar chemical reagents.

24. An apparatus for combining chemical reagents as claimed in claims (14) or (18) substantially 100 as herein described with reference to Figures 1, 2, 4, 5 and 6 of the accompanying drawings.

25. An apparatus for combining chemical reagents as claimed in claims (14) or (18) substantially 105 as herein described with reference to Figures 3, 7, 8 and 9 of the accompanying drawings.

New claims or amendments to claims filed on
21.6.82
Superseded claims 1-25

110 New or amended claims:-

1. A method of combining chemical reagents by:
1. dispensing two or more reagents, optionally in a solvent phase into a conduit system in the form of 115 discrete volumes of liquid, each discrete volume being separated from each other by an inert immiscible liquid and,
2. moving the immiscible liquid and discrete volumes through the conduit system in such a 120 manner that predetermined discrete volumes coalesce with each other thereby combining the reagents.

2. A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.

3. A method as claimed in claim (1) in which the discrete volumes of chemical reagents are large enough to be elongated by the walls of the conduits.

130 4. A method as claimed in claims (1), (2) and 3 in

which each of the discrete volumes of chemical reagents is formed by the passing of the chemical reagent from a conduit into one or both of two conduits which have previously been filled with the inert immiscible liquid and which converge with the conduit containing the chemical reagent, followed by the moving of the inert immiscible liquid causing a segment of the chemical reagent to become separated from the chemical reagent which remains in the duct from which the chemical reagent was introduced.

5. A method as claimed in claimed in claim (4) in which the conduit from which the chemical reagent is introduced has a constriction at the point where it 15 meets the two conduits which were previously filled with inert immiscible liquid, which allows the segment of the liquid to be mixed to be separated by a less powerful movement of the inert immiscible liquid, and with a more predictable volume.

20 6. A method as claimed in claim (4) in which the volume of the chemical reagent is estimated before separation by assuming said volume is spherical and measuring the diameter of such a sphere with a microscope of telescope with a graduated eyepiece, 25 after which an adjustment of said volume may be made and said volume separated.

7. A method as claimed in claim (4) in which the volume of the chemical reagent that is passed into the conduits which were previously filled with inert 30 immiscible liquid is sufficiently large that said volume occupies a length of said conduits, which length may be used as a measure of said volume prior to the adjustment and separation of said volume.

35 8. A method as claimed in claim (4) in which the volume of the chemical reagent that is passed into the conduits that were previously filled with inert immiscible liquid is determined by displacing the required volume out of the conduit containing the 40 chemical reagent by moving a syringe or piston a known distance, or by means of a well calibrated pump.

9. A method as claimed in any preceding claim in which the passage of a segment of chemical reagent 45 is preceded by the passage of a segment of cleansing liquid which is miscible with the reagents, but immiscible with the inert immiscible liquid.

10. A method as claimed in any previous claim in which surface acting chemical agents are dissolved 50 in the chemical reagents or in the immiscible liquid or in both.

11. A method as claimed in claim (1) in which the discrete volumes of the chemical reagents are replaced by discrete volumes of liquids containing 55 suspended biological micro-organisms.

12. A method of combining chemical reagents as claimed in claim (1) substantially as herein described with reference to Figures 3, 7, 8 and 6 of the accompanying drawings.

60 13. A method of combining chemical reagents as claimed in claim (1) substantially as herein described with reference to Figures 3, 7, 8 and 9 of the accompanying drawings.

14. An apparatus for carrying out any of the 65 methods claimed in previous claims comprising two

plates held in face to face contact, in which one or both plates possesses a set of indentations on the surface to be mated such that closed conduits are formed, said conduits being in communication with reservoirs of at least 2 reagents and an immiscible liquid.

15. An apparatus as claimed in claim (1) in which valves, pumps, syringes, pistons or flexible sheets or tubes are used to produce and control the movements of the liquid components of the system.

16. An apparatus as claimed in claim (14) in which at least one of the plates is transparent.

17. An apparatus as claimed in claims (14), (15) or (16) having three or more plates which allows the 80 construction of overlapping and crossing over conduits.

18. An apparatus for carrying out the method claimed in claim (1) in which tubular elements are joined so as to form the conduits for the introduction of discrete volumes of at least two chemical reagents, and zones where at least three conduits meet, which zones allow the coalescence of said discrete volumes of chemical reagents.

19. An apparatus as claimed in claims (14) or (18) 90 which possesses one or more zones for the coalescence of discrete volumes of chemical reagents, each zone being characterized by the meeting of at least three conduits, preferably in a 'Y' or 'T' configuration in the case of three conduits, and in which each 95 conduit may be of a different width.

20. An apparatus as claimed in claims (14) or (18) which possesses one or more zones for the coalescence of discrete volumes of chemical reagents, each zone being characterized by a constriction in a 100 conduit, which constriction is bypassed by a conduit which has a diameter comparable to that of the constriction and which runs from shortly before the constriction to shortly after it.

21. An apparatus as claimed in claims (14) or (18) 105 which possesses one or more zones for the coalescence of discrete volumes of chemical reagents, each zone being characterized by a depression in which a discrete volume of chemical reagent is held by gravity while another discrete volume is moved by 110 the inert immiscible liquid towards a nearby opening, which movement causes the discrete volumes to become pressed against each other allowing coalescence.

22. An apparatus as claimed in (14) or (18) 115 possessing conduits with hydrophobic walls for use with aqueous or polar chemical reagents.

23. An apparatus as claimed in (14) or (18) possessing conduits with hydrophilic walls for use with hydrophilic or non-polar chemical reagents.

24. An apparatus for combining chemical reagents as claimed in claims (14) or (18) substantially as herein described with reference to Figures 1, 2, 4, 5 and 6 of the accompanying drawings.

25. An apparatus for combining chemical reagents as claimed in claims (14) or (18) substantially as herein described with reference to Figures 3, 7, 8 and 9 of the accompanying drawings.

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